studies that will hopefully bring us to the point that we can apply precision medicine to the therapy of multiple myeloma.

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# RASAL2: Wrestling in the Combat of Ras Activation

**Jia Shen,[1,2] Yan Wang,[1] and Mien-Chie Hung[1,2,3,*]**
[1]Department of Molecular and Cellular Oncology, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030, USA
[2]The University of Texas Graduate School of Biomedical Sciences at Houston, Houston, TX 77030, USA
[3]Center for Molecular Medicine and Graduate Institute of Cancer Biology, China Medical University, Taichung 402, Taiwan
*Correspondence: mhung@mdanderson.org
http://dx.doi.org/10.1016/j.ccr.2013.08.024

**Oncogenic activation of Ras proteins due to missense mutations is frequently detected in human cancers but rarely in breast cancer. In this issue of *Cancer Cell*, McLaughlin and colleagues report that ablation of the GasGAP gene, *RASAL2*, is an alternative mechanism by which Ras becomes activated in breast cancer.**

Ras GTPases are essential components of signaling pathways that emanate cues from cell surface receptors to regulate diverse cellular processes, including cell cycle progression, cell survival, actin cytoskeletal organization, cell polarity and movement, as well as vesicular and nuclear transport (Vigil et al., 2010). Ras proteins (H-Ras, N-Ras, and K-Ras), together with their two key regulators (guanine nucleotide exchange factors/GEFs and GTPase-activating proteins/GAPs), constitute cellular binary switches that cycle between "on" and "off" conformations conferred by the loading of GTP or GDP, respectively. The transition between the active GTP-bound and inactive GDP-bound states of Ras GTPases is controlled by GEFs, which promote the activation of Ras proteins by stimulating GDP for GTP exchange, and GAPs, which terminate the activation status by accelerating Ras-mediated GTP hydrolysis (Bos et al., 2007; Tcherkezian and Lamarche-Vane, 2007). Ras GTPases and cancer are tightly associated, as high frequency of mutational activation of Ras proteins is observed in ~33% of human cancers. The intrinsic GTP hydrolysis activity of Ras is the predominant target of the most common somatic mutations that are found in the oncogenic variants of *RAS* alleles (Pylayeva-Gupta et al., 2011). Specifically, oncogenic substitution in residue G12 or G13 results in pronounced attenuation of intrinsic GTP hydrolysis, which leads to the persistence of the GTP-bound state of Ras and subsequently activates a multitude of Ras-dependent downstream effector pathways. Beyond Ras, hyperactivation of GEFs and functional deregulation, including suppression and loss-of-function mutations of GAPs, have also been suggested to play important roles in cancer progression (Vigil et al., 2010).

Despite the prevalence of oncogenic RAS mutations in human cancers, *K-RAS*, *H-RAS*, and *N-RAS* are rarely mutated in breast cancer (Karnoub and Weinberg, 2008). Nonetheless, the Ras/ERK pathway is hyperactivated in more than half of breast cancers and has been implicated in tumor progression and recurrence (von Lintig et al., 2000), suggesting that Ras proteins may be more frequently activated by alternative mechanisms in this type of tumors. A new study by McLaughlin et al. (2013) in this issue of *Cancer Cell* uncovers the role of RASAL2,

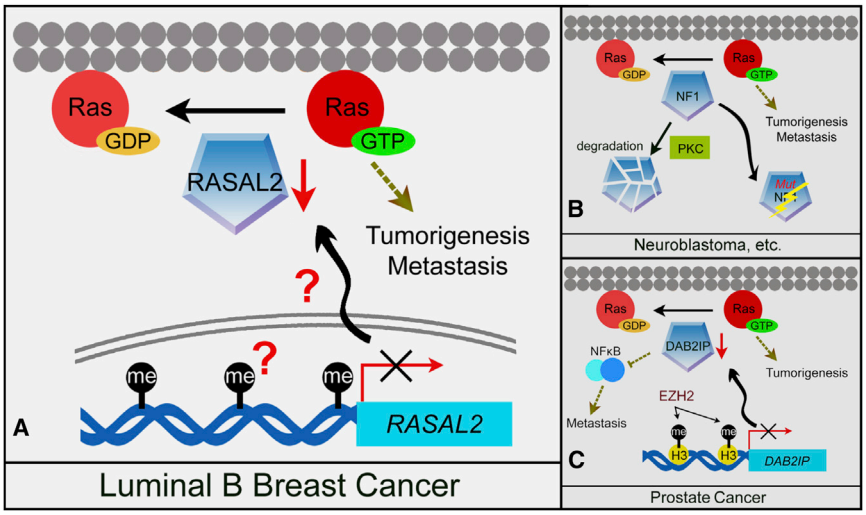


**Figure 1. Alternative Activation of Wild-Type Ras via Deregulation of RasGAPs in Human Cancers**

(A) Suppression of *RASAL2* is specifically found in human luminal B breast cancer and correlates with the hypermethylation status of *RASAL2* promoter CpG sites. Loss of *RASAL2* activates wild-type Ras and promotes cancer progression and metastasis in the breast cancer xenograft model and genetically engineered mouse models (McLaughlin et al., 2013).

(B) Germline mutational loss of the NF1 tumor suppressor is found in familial cancer syndrome neurofibromatosis type 1 and is mutated or suppressed via PKC-dependent proteasome degradation in several sporadic cancers, including glioblastoma, non-small cell lung cancer, neuroblastoma, and melanoma (McGillicuddy et al., 2009; Vigil et al., 2010).

(C) DAB2IP, another RasGAP, is also epigenetically silenced by EZH2 and functions as a tumor and metastasis suppressor via activation of both Ras and NF-kB pathways in prostate cancer (Min et al., 2010).

a RasGAP that is less well characterized, as a tumor suppressor in breast cancer through direct blockage of Ras activation. They found that *RASAL2* is frequently absent or minimally expressed in cells derived from luminal breast cancer. Reconstitution of RASAL2 in cells with low endogenous expression suppressed Ras-GTP and the activation of downstream ERK. Conversely, knockdown of RASAL2 in normal immortalized mammary epithelial cells enhanced Ras-GTP and increased the level of phospho-ERK. These results demonstrate that RASAL2 is a functional RasGAP and that its loss activates the Ras/ERK signaling pathway.

Whereas reconstitution of RASAL2 in *RASAL2*-deficient breast cancer cells inhibited anchorage-independent colony growth and potently suppressed tumor growth in vivo, it had almost no significant effect on RAS mutant tumors. The dependency of Ras mutation status suggests that the RasGAP domain in RASAL2 plays an important role. Indeed, two RasGAP domain mutants (K417E and K567X) that are defective in blocking the activation of Ras/ERK pathway failed to suppress anchorage-independent cell growth, indicating that the RasGAP domain is essential for the tumor suppressor function of RASAL2. Elevated levels of K-Ras-GTP and H-Ras-GTP significantly contributed to the pathogenesis promoted by RASAL2 inactivation. In contrast, ablation of RASAL2 enhanced cell migration and invasion in vitro and promoted cancer progression from ductal carcinoma in situ (DCIS) to invasive carcinoma in a breast cancer xenograft model using MCF10A-DCIS cells.

The functional role of RASAL2 was further examined by the authors in genetically engineered mice lacking *Rasal2*. *Rasal2*$^{-/-}$ mice exhibit shorter overall survival as compared to control animals. However, *Rasal2*$^{-/-}$ mice did not develop tumors, suggesting that the loss of *Rasal2* itself is not sufficient to drive tumorigenesis in mice. Interestingly, *Rasal2*$^{-/-}$ mice that crossed with *MMTVneu* developed substantially more metastases than the *MMTVneu* mice, with higher frequency and larger size of metastases, as well as an additional spectrum of distant metastases, including brain, kidney, ovary, and gastrointestinal tract, which are not observed in *MMTVneu* mice. Correspondingly, the activation of Ras/ERK and AKT were substantially elevated in the compound mice, suggesting that the loss of *Rasal2* promotes metastasis via activation of Ras. In addition, the authors also propose that RASAL2 may play a broader tumor-suppressive role in other cancer types as the loss of *Rasal2* potently promotes metastasis in *Trp53* mutant mice.

To understand the suppression mechanism of RASAL2 in human breast cancers, McLaughlin et al. (2013) examined the DNA methylation status of *RASAL2* promoter and identified two CpG sites that are specifically methylated in luminal B breast tumors, revealing an alternative mechanism of Ras activation through epigenetic ablation of *RASAL2* (Figure 1A). Notably, the DNA methylation patterns are significantly different between luminal B and basal-like breast tumors (Holm et al., 2010), with one being the most and the other least frequently methylated, respectively. As little is known about the molecular mechanisms that are responsible for the suppression of *RASAL2* via promoter methylation, particularly in luminal B breast cancer, more questions remain, such as whether methylation of *RASAL2* promoter CpG sites is a consequence of generally elevated DNA methylation due to enhanced activity of DNA methyltransferases in luminal B subtype or whether *RASAL2* is specifically targeted for silencing to activate Ras/ERK signaling in these tumors. The detailed contribution of promoter methylation to the suppression of *RASAL2* in breast cancer will be interesting to further pursue. The evidence shown by the authors provides an interesting correlation between enhanced promoter methylation and reduced *RASAL2* mRNA levels, but it is still not clear if other mechanisms for *RASAL2* inactivation coexist in breast cancers. Indeed, RasGAPs, exemplified by NF1 and DAB2IP tumor suppressors, are susceptible to multiple ways of deregulation (Figures 1B and 1C), including epigenetic silencing, proteasome-mediated degradation, and loss-of-function mutations (Bos et al., 2007; McGillicuddy et al., 2009; Min et al., 2010). Understanding the exact regulations of *RASAL2*

suppression could shed light on new anticancer strategies that inhibit the activation of wild-type Ras by restoring expression of the corresponding RasGAP.

Intriguingly, despite the fact that each of the RasGAP members harbors a conserved RasGAP domain, not all exhibit tumor suppressor functions (Min et al., 2010). Accordingly, the existence of non-Ras-associated functions of RasGAPs has been proposed and further demonstrated in the case of DAB2IP (Min et al., 2010), which acts as a signaling scaffold that coordinately regulates Ras and NF-kB activation to promote tumor growth and metastasis (Figure 1C), respectively. Whether other conserved domain(s) besides the RasGAP domain of RASAL2 also exert a Ras-independent tumor-suppressive signaling cascade remains to be investigated. Additionally, how the RasGAPs coordinate with each other to regulate the activation of Ras in different human cancers is not clear. Epigenetic suppression of DAB2IP (Dote et al., 2004) and the mutation of NF1 (The Cancer Genome Atlas Database) are also observed in breast cancers, raising the question of whether the deregulation events of RasGAPs are mutually exclusive or whether they coexist for sufficient addiction to wild-type Ras activation in breast cancer.

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# It Takes a CAD to Kill a Tumor Cell with a LMP

**Erich Gulbins[1] and Richard N. Kolesnick[2,*]**
[1]Department of Molecular Biology, University of Duisburg-Essen, Hufelandstrasse 55, 45122 Essen, Germany
[2]Laboratory of Signal Transduction, Memorial Sloan-Kettering Cancer Center, 415 East 68th Street, New York, NY 10065, USA
*Correspondence: r-kolesnick@ski.mskcc.org
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**Cancer cells display lysosome hypertrophy, secreting lysosomal hydrolases for tumor progression. Hypertrophy renders lysosomes fragile, increasing lysosomal membrane permeabilization (LMP) tendency. In this issue of *Cancer Cell*, Petersen and colleagues show that lysosomal sphingomyelin content determines LMP and cationic drugs displace acid sphingomyelinase from lysosomal membranes, increasing tumor LMP and death.**

Early on after the discovery of lysosomes by Christian de Duve as a separate compartment that confines highly destructive hydrolases for the demolition and reutilization of cellular substituents, the concept that these structures might alternatively represent "suicide bags" was proposed (de Duve, 1983). This led to an intense search for lysosomatrophic agents that might access this biology for therapeutic purpose. Although a set of lysosomal detergents with long hydrophobic tails and medium pK were defined as capable of inducing lysosomal membrane permeabilization (LMP) and thereby releasing the destructive power of hydrolases into the cytoplasm, this concept was rapidly retired, because it was not possible to assign lethal causality to these compounds based on LMP as opposed to postmortal lysosomal destruction (Miller et al., 1983).

In the interim, the field of cathepsin protease biology developed. Cathepsins represent a class of cysteine, serine, and aspartate proteases that segregate into lysosomes and, under homeostatic conditions, serve to reutilize polypeptides for ongoing cellular metabolic requirements. Lysosomes contain more than 50 cathepsins, and, as a class, they have been associated with various human pathologies, including cancer. Numerous cathepsins and other lysosomal enzymes, e.g., heparanase, have been strongly associated with cancer cell proliferation, angiogenesis, and metastasis (Kallunki et al., 2013). Evidence indicates that, upon secretion, these tumor-promoting lysosomal enzymes act extracellularly. To accommodate this burgeoning need, tumor cells in general display enlargement